Programmable RNA Tracking in Live Cells with CRISPR/Cas9.

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Public Summary:
We have hijacked the Crispr/Cas system for genome engineering to enable live cell RNA recognition and tracking. Here, we demonstrate that nuclease-inactive S. pyogenes CRISPR/Cas9 can bind RNA in a nucleic-acid-programmed manner and allow endogenous RNA tracking in living cells. We show that nuclear-localized RNA-targeting Cas9 (RCas9) is exported to the cytoplasm only in the presence of sgRNAs targeting mRNA and observe accumulation of ACTB, CCNA2, and TFRC mRNAs in RNA granules that correlate with fluorescence in situ hybridization. We also demonstrate time-resolved measurements of ACTB mRNA trafficking to stress granules. Our results establish RCas9 as a means to track RNA in living cells in a programmable manner without genetically encoded tags. This has much potential as we can now modify RNA in live cells.

Scientific Abstract:
RNA-programmed genome editing using CRISPR/Cas9 from Streptococcus pyogenes has enabled rapid and accessible alteration of specific genomic loci in many organisms. A flexible means to target RNA would allow alteration and imaging of endogenous RNA transcripts analogous to CRISPR/Cas-based genomic tools, but most RNA targeting methods rely on incorporation of exogenous tags. Here, we demonstrate that nuclease-inactive S. pyogenes CRISPR/Cas9 can bind RNA in a nucleic-acid-programmed manner and allow endogenous RNA tracking in living cells. We show that nuclear-localized RNA-targeting Cas9 (RCas9) is exported to the cytoplasm only in the presence of sgRNAs targeting mRNA and observe accumulation of ACTB, CCNA2, and TFRC mRNAs in RNA granules that correlate with fluorescence in situ hybridization. We also demonstrate time-resolved measurements of ACTB mRNA trafficking to stress granules. Our results establish RCas9 as a means to track RNA in living cells in a programmable manner without genetically encoded tags.